CHAPTER IV
RESULTS

1. Renal NOS Protein Expression

The expression of eNOS protein in renal tissues detected by immunohistochemical method is shown in Figures 4 – 6. In the sham group (Figure 4A), the expression occurred in both glomeruli and renal tubular epithelium. The staining of eNOS protein was present in cortex as well as medulla. One day after UUO caused an increase of eNOS protein expression in both cortex and medulla (Figure 4B). The intensity score increased from 1 to be 2 (Table 1). In addition, this alteration was similarily observed in 7-day duration after UUO in which the score rose to be 2 (Table 1 and Figure 4C).

In the 1-day UUO group, treatment with either ACEI or ARA could slightly decreased renal eNOS protein expression in both cortex and medulla (Figures 5 and 6, respectively). The intensity scores were 1-2 (Table 1). However, administration of either ACEI or ARA had no effect on the expression of eNOS in the cortex area (Figure 7). Interestingly, in 7-day UUO rats, the eNOS protein expression in the medulla rose from the scores of 2 to be 3 (Table 1 and Figure 8C & D) after ACEI or ARA treatments.

The right kidney, non obstruction, demonstrated the eNOS protein expression in the same patterns as the sham group (Table 1).

Regard to iNOS, both 1-day and 7-day UUO condition in the present study did not influence the protein expression (data not shown).

Table 1 The intensity scores of renal eNOS protein expression in cortex and medulla from left (obstruction) and right (non obstruction) kidney of rats in Sham, UUO, UUO+ACEI, and UUO+ARA after 1 day or 7 days post UUO.

Groups		Duration period after UUO			
		1 day		7 days	
		Cortex	Medulla	Cortex	Medulla
Sham	Lt.	1	1	1	1
	Rt.	1	1	1	1
UUO	Lt.	2	2	2	2
	Rt.	1	1	1	1
UUO + ACEI	Lt.	1-2	1-2	2	3
	Rt.	1	1	1	1
UUO + ARA	Lt.	1-2	1-2	2	3
	Rt.	1	1	1	1

Renal eNOS protein expression was assessed by immunohistochemistry. The intensity of eNOS staining was scored from 0 to 3 (0 = no staining, 1 = weak positive, 2 = moderate staining, and 3 = strongly positive staining) (n = 4 – 5/group). All slides were viewed and scored by three blinded observers. (UUO = Unilateral Ureteral Obstruction, ACEI = angiotensin converting enzyme inhibitor, ARA = angiotensin II receptor type 1 antagonist, Lt. = left kidney, Rt. = right kidney).

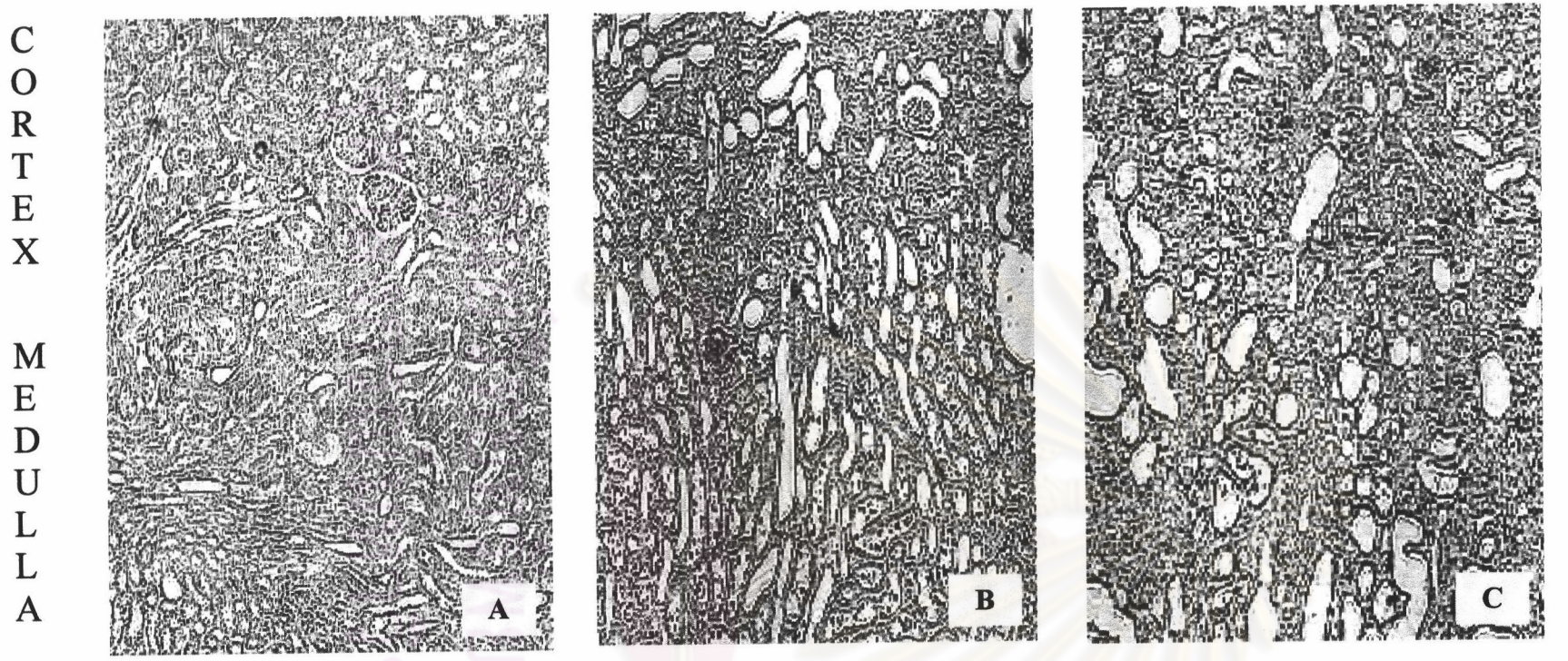

Figure 4 Immunohistochemical staining of renal eNOS protein expression. A: Sham (C = 1, M = 1), B: 1 day after UUO (C = 2, M = 2), and C: 7 days after UUO (C = 2, M = 2). Intensity scores are presented in parenthesis (C = cortex, M = medulla). Original magnification: 40X.

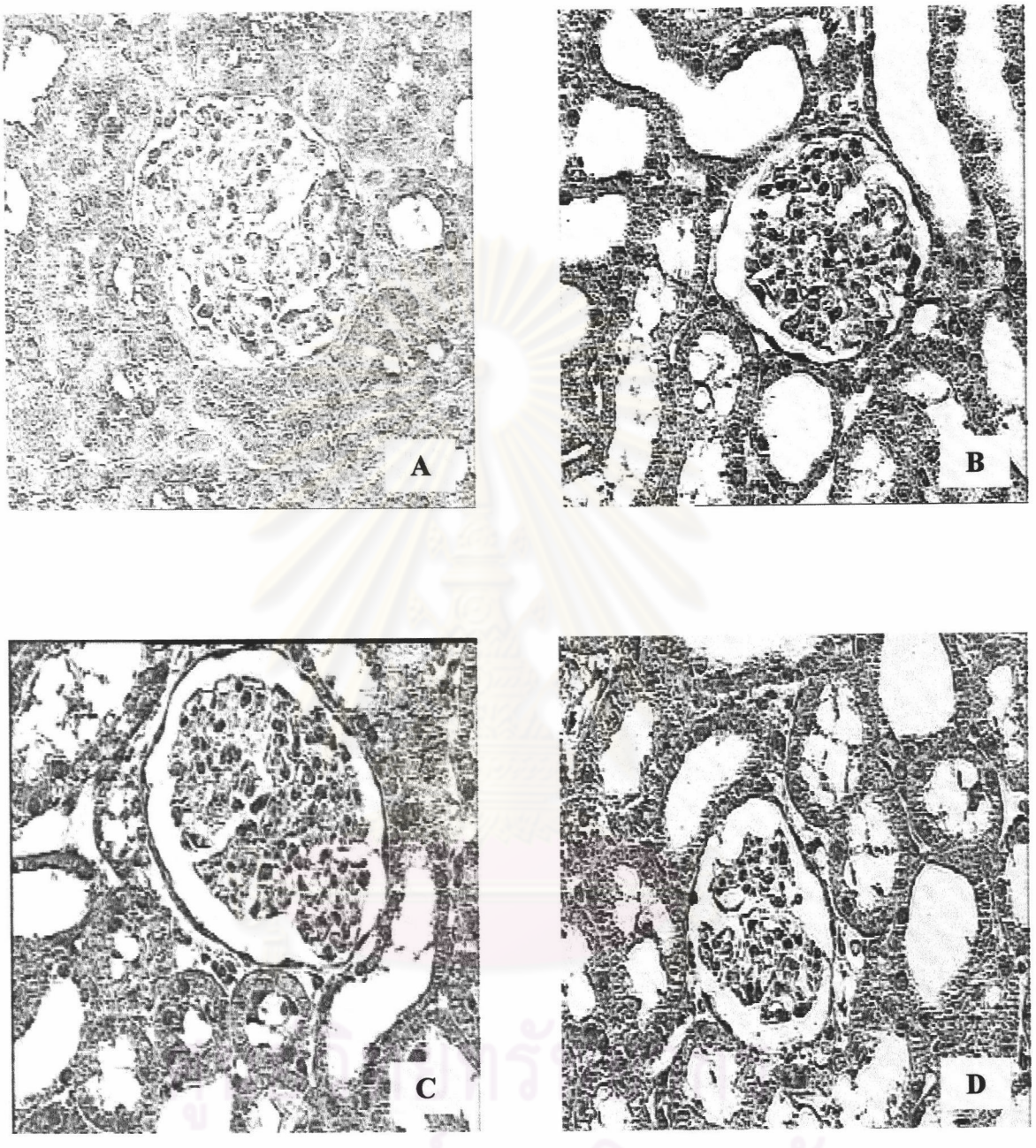

Figure 5 Immunohistochemical staining of renal eNOS protein expression in cortex from 1 - day UUO groups. A: Sham (1), B: UUO (2), C: UUO+ACEI (1-2), and D: UUO+ARA (1-2). Intensity scores are presented in parenthesis. Original magnification: 200X.

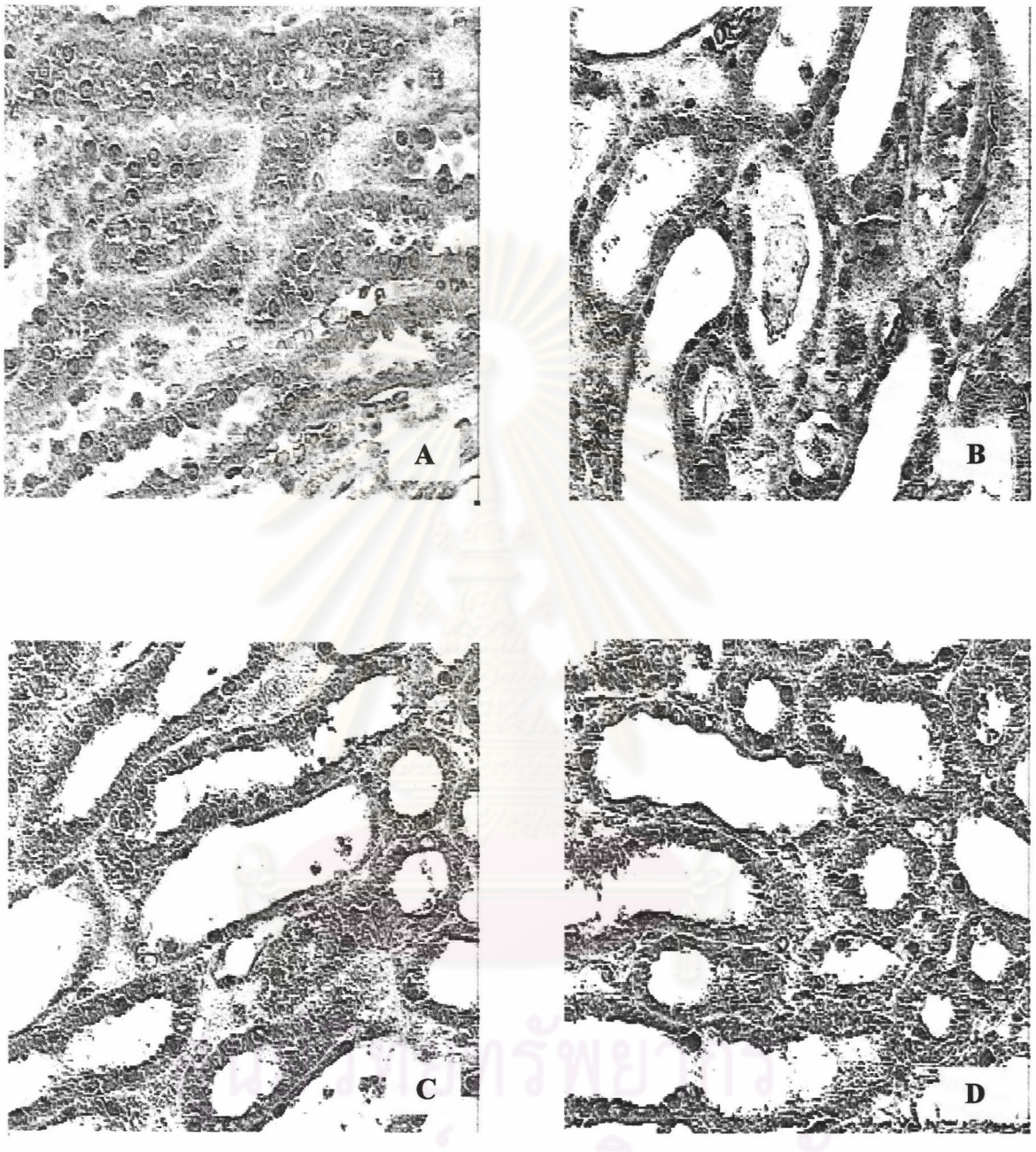

Figure 6 Immunohistochemical staining of renal eNOS protein expression in medulla from 1 - day UUO groups . A: Sham (1), B: UUO (2), C: UUO+ACEI (1-2), and D: UUO+ARA (1-2). Intensity scores are presented in parenthesis. Original magnification: 200X.

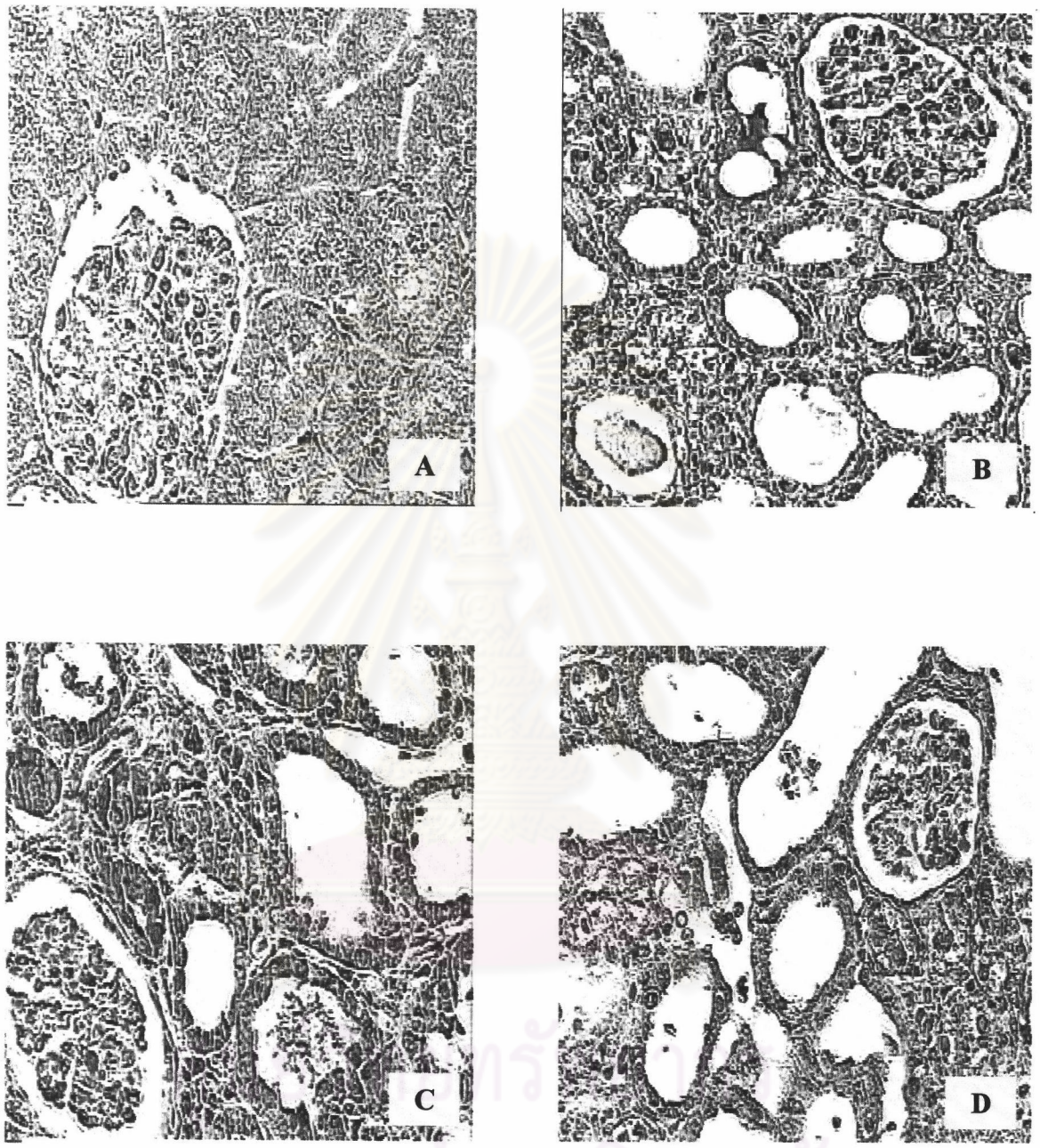

Figure 7 Immunohistochemical staining of renal eNOS protein expression in cortex from 7-day UUO groups. A: Sham (1), B: UUO (2), C: UUO+ACEI (2), and D: UUO+ARA (2). Intensity scores are presented in parenthesis. Original magnification: 200X.

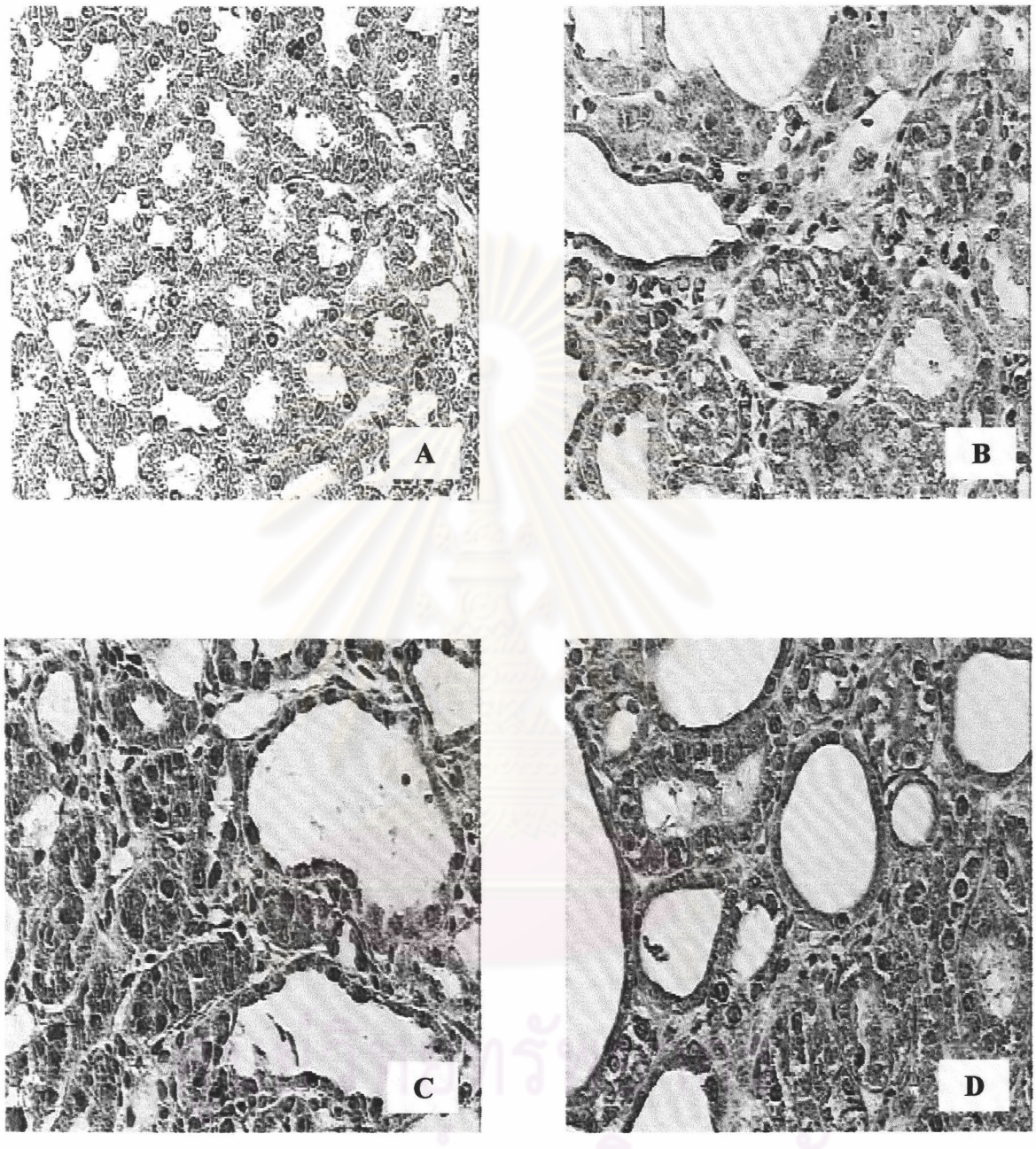

Figure 8 Immunohistochemical staining of renal eNOS protein expression in medulla from 7-day UUO groups. A: Sham (1), B: UUO (2), C: UUO+ACEI (3), and D: UUO+ARA (3). Intensity scores are presented in parenthesis. Original magnification: 200X.

2. Nitric Oxide Production

The stable metabolite of NO, nitrite, was utilized to determine NO production. As shown in Figure 9, serum nitrite concentrations significantly rose from 11.80 ± 0.72 μmol/L in the sham group to 28.74 ± 3.99 μmol/L ($p < 0.05$) in the 1-day UUO group and increased from 13.21 ± 0.18 to 30.10 ± 2.20 μmol/L ($p < 0.05$) in the 7-day UUO rats. Treatment with ACEI as well as ARA could normalize the heightened nitrite levels induced by UUO in both 1-day and 7-day groups to the levels of sham animals.

3. Histopathological Study

The pathological study in renal tissue was defined by histological examination and shown in Figures 10 to 14. One day after UUO, the kidney showed moderate dilatation (scores 2-3; Table 2) of tubule with a few cast formation and mild brush border membrane loss (Figure 10B). In addition, some interstitial fibrosis and cell infiltration also occurred in both cortex and medulla (Figures 11B and 12B). Treatments with either ACEI (Figures 11C and 12C) or ARA (Figures 11D and 12D) in 1-day UUO rats could attenuate the pathological changes.

In the longer period, 7-day UUO, progressive morphological changes were observed (scores 3, Table 2). There was more dilation of Bowman's capsule space with a mild glomerular damage (Figure 13B). The size of glomerulus was compressed while the collecting ducts and distal tubules were much more expanded (Figure 13B). Interstitial fibrosis and cell infiltration were prominent (Figures 13B and 14B). Treatment with ACEI (Figures 13C and 14C) had more effectiveness in reduction of tissue damage than ARA (Figures 13D and 14D, Table 2).

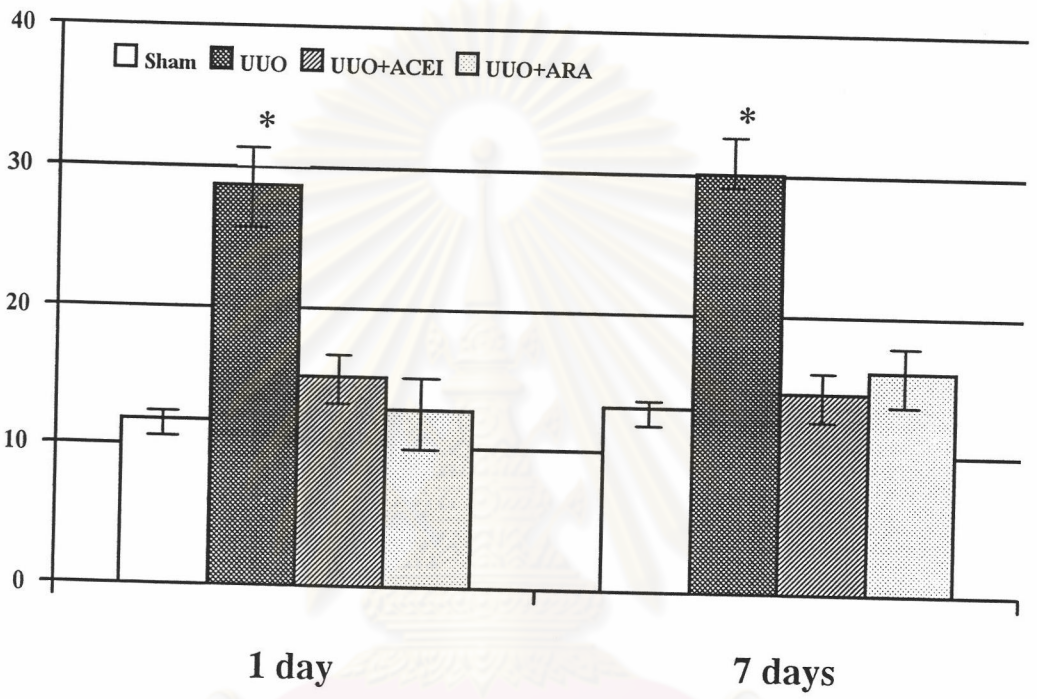

Figure 9 Serum level of nitrite concentration in Sham, UUO, UUO+ACEI and UUO+ARA groups from 1 day and 7 days after UUO (n = 6±1/group). * $p<0.05$ vs. Sham; UUO+ACEI; UUO+ARA groups both 1 day and 7 days.

Table 2 The pathological scores of renal cortex and medulla from left (obstruction) and right (non obstruction) kidney of rats in Sham, UUO, UUO+ACEI, and UUO+ARA after 1 day or 7 days post UUO.

Groups		Duration period after UUO			
		1 day		7 days	
		Cortex	Medulla	Cortex	Medulla
Sham	Lt.	0-1	0-1	0-1	0-1
	Rt.	0-1	0-1	0-1	0-1
UUO	Lt.	2-3	2-3	3	3
	Rt.	0-1	0-1	0-1	0-1
UUO + ACEI	Lt.	1-2	1-2	1-2	1-2
	Rt.	0-1	0-1	0-1	0-1
UUO + ARA	Lt.	1-2	1-2	2-3	2-3
	Rt.	0-1	0-1	0-1	0-1

Renal tissue injury was assessed in PAS and Masson's trichrome technique stained tissue sections. Sections were scored in blinded, semiquantiative manner. (n = 4-5/group) The numerical scores indicate the following: 0 = normal structure; 1 = areas of tubular epithelial cell swelling, vacuolar degeneration, necrosis, and desquamation involving less than 25% of cortical tubules; 2 = similar changes involving greater than 25% but less than 50% of cortical tubules; 3 = similar changes involving greater than 50% but less than 75% of cortical tubules; 4 = similar changes involving greater than 75% of cortical tubules, and 5 = complete cortical necrosis. (UUO = unilateral ureteral obstruction, ACEI = angiotensin converting enzyme inhibitor, ARA = angiotensin II receptor type 1 antagonist, Lt. = left kidney, Rt. = right kidney).

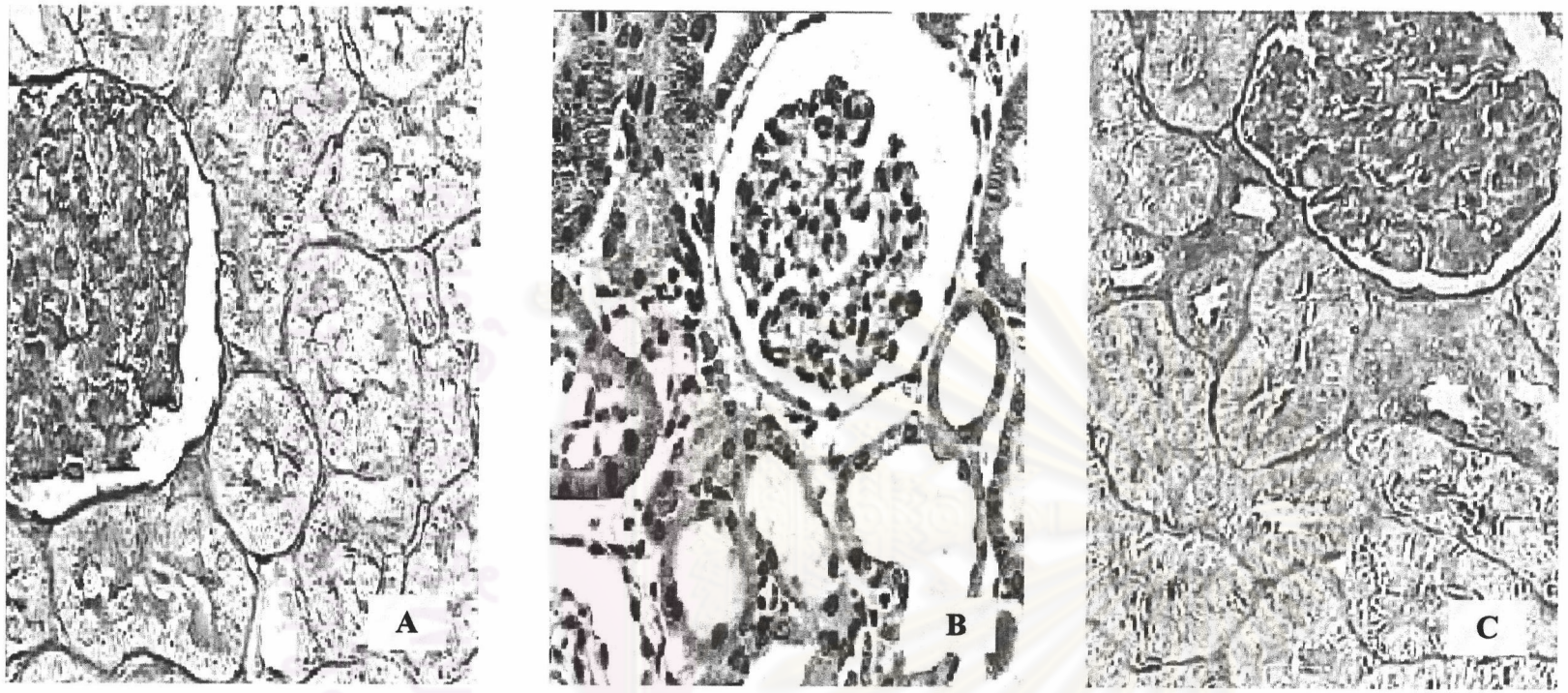

Figure 10 Representative PAS-staining tissue sections of renal cortex A: Sham (C = 1), B: 1 day after UUO: left (obstruction) kidney(C = 2), and C: 1 day after UUO: right (non obstruction) kidney (C = 1). Pathological scores are presented in parenthesis. Original magnification: 200X.

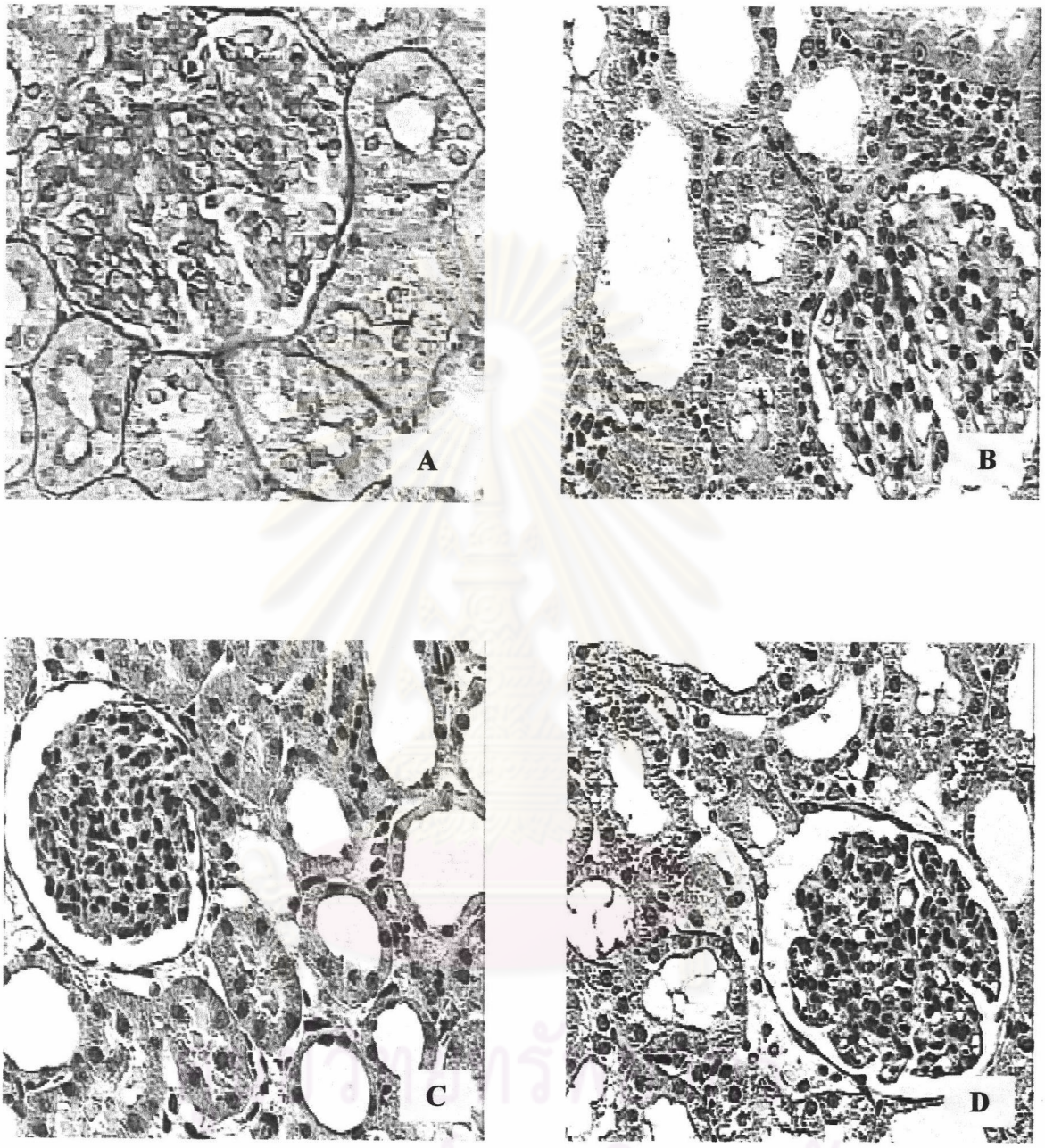

Figure 11 Representative PAS-staining tissue sections in renal cortex of left (obstruction) kidney from 1 day after UUO groups. A: Sham (0-1), B: UUO (2-3), C: UUO+ACEI (1-2), and D: UUO+ARA (1-2). Pathological scores are presented in parenthesis. Original magnification: 200X.

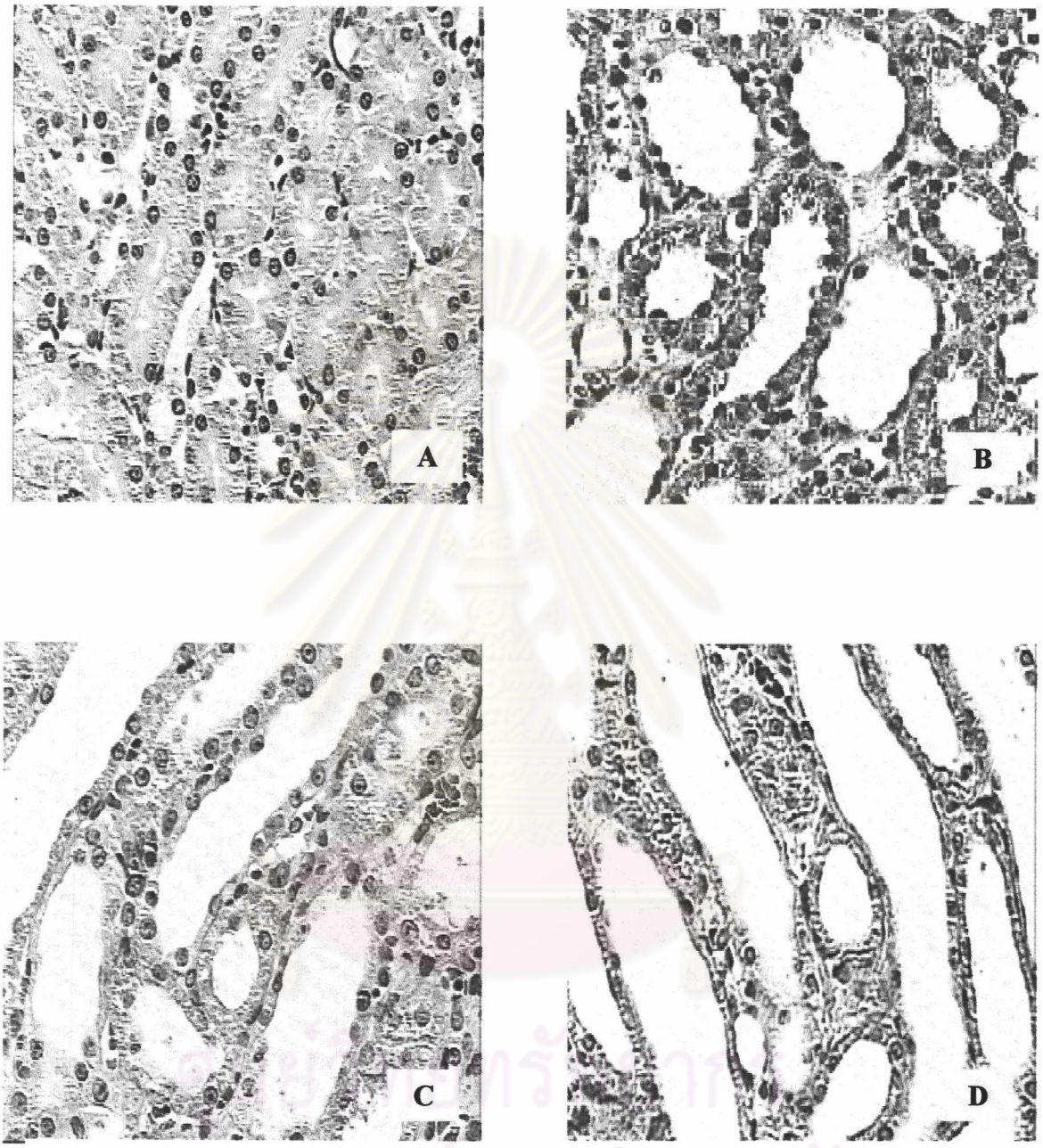

Figure 12 Representative PAS-staining tissue sections in renal medulla of left (obstruction) kidney from 1 day after UUO groups. A: Sham (0-1), B: UUO (2-3), C: UUO+ACEI (1-2), and D: UUO+ARA (1-2). Pathological scores are presented in parenthesis. Original magnification: 200X.

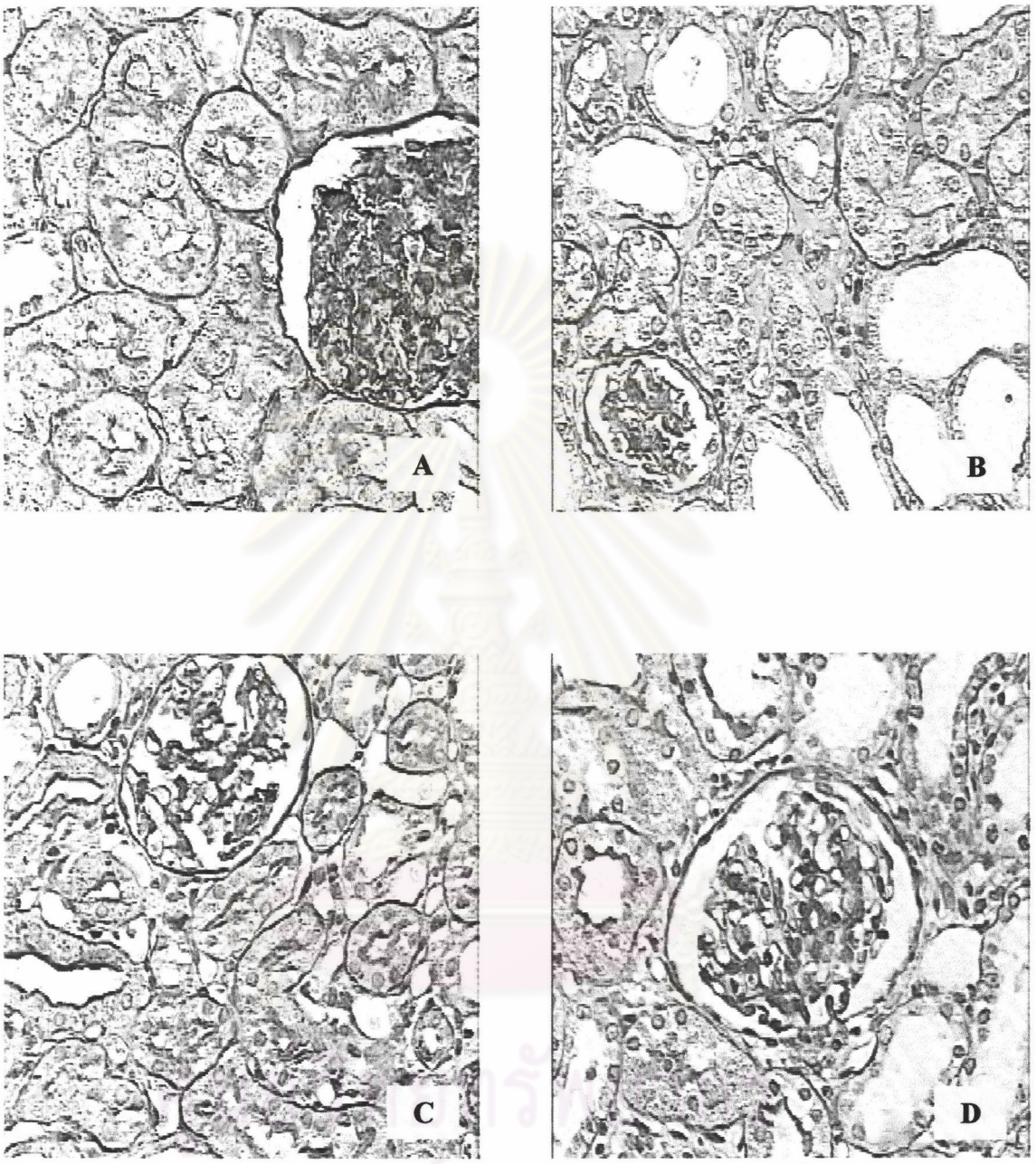

Figure 13 Representative PAS-staining tissue sections in renal cortex of left (obstruction) kidney from 7 day after UUO groups. A: Sham (0-1), B: UUO (3), C: UUO+ACEI (1-2), and D: UUO+ARA (2-3). Pathological scores are presented in parenthesis. Original magnification: 200X.

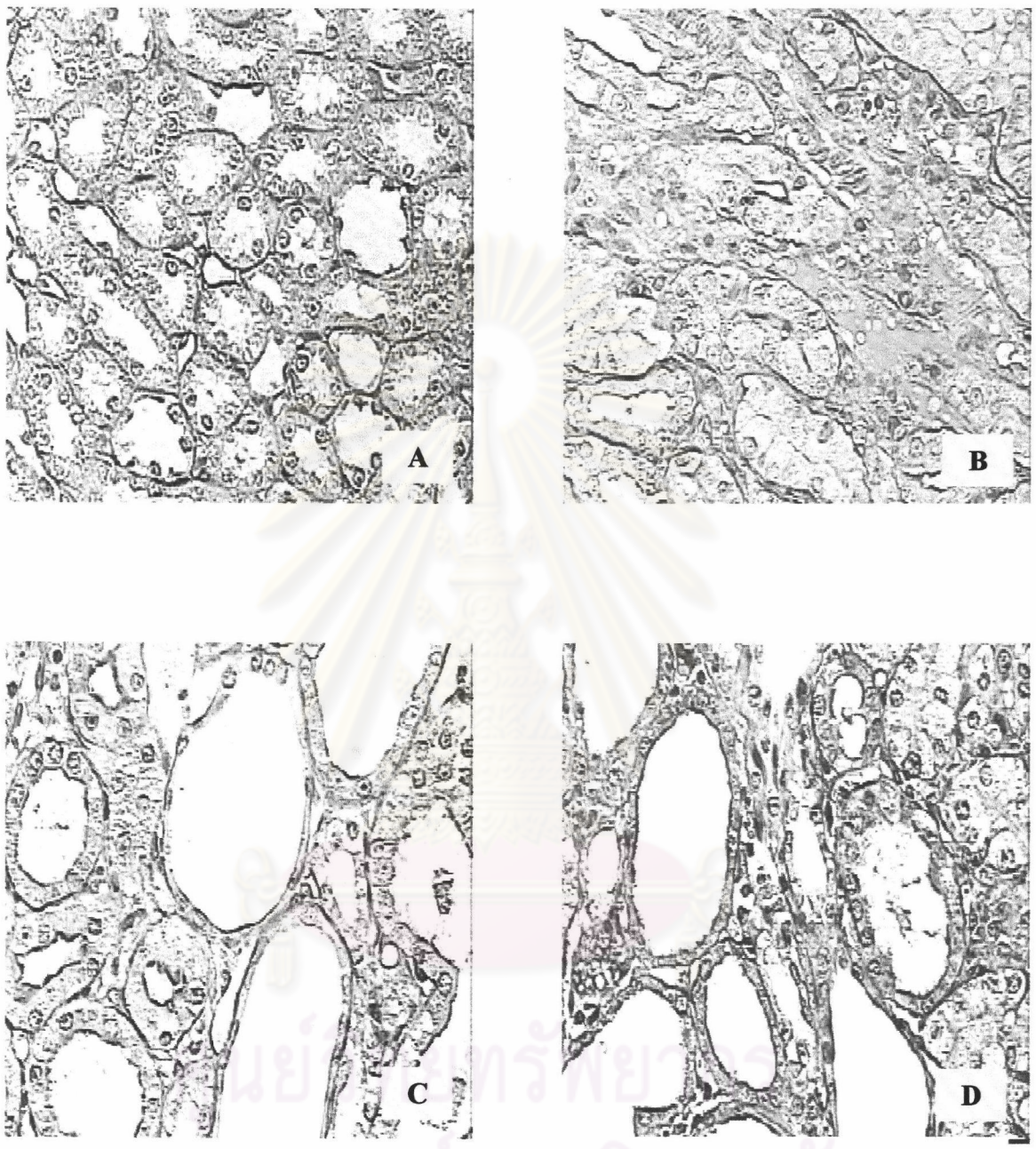

Figure 14 Representative PAS-staining tissue sections in renal medulla of left (obstruction) kidney from 7 day after UUO groups. A: Sham (0-1), B: UUO (3), C: UUO+ACEI (1-2), and D: UUO+ARA (2-3). Pathological scores are presented in parenthesis. Original magnification: 200X.

As predicted, the right kidney (non obstruction) of rats undergone UUO had normal histological study at both 1 day and 7 days after UUO (data not shown).

4. Fractional Excretion of Electrolytes

After 1 day and 7 days post UUO, the fractional excretion of Na^+ (FE_{Na+}), K^+ (FE_{K+}), and Cl^- (FE_{Cl}^-) of right (non obstruction) in all groups were comparable and not significanly different from those of the sham animals (Figure 15).

5. Metabolic Parameters in Serum and Renal Function

Serum concentrations of Na^+, K^+, Cl^-, Cr, and BUN in all groups studied showed comparable levels (Table 3). Moreover, creatinine clearance and urine flow rate were not significantly altered by UUO or treatment with either ACEI or ARA (Table 3). No significant differences in all these parameters were observed between 1-day and 7-day studies.

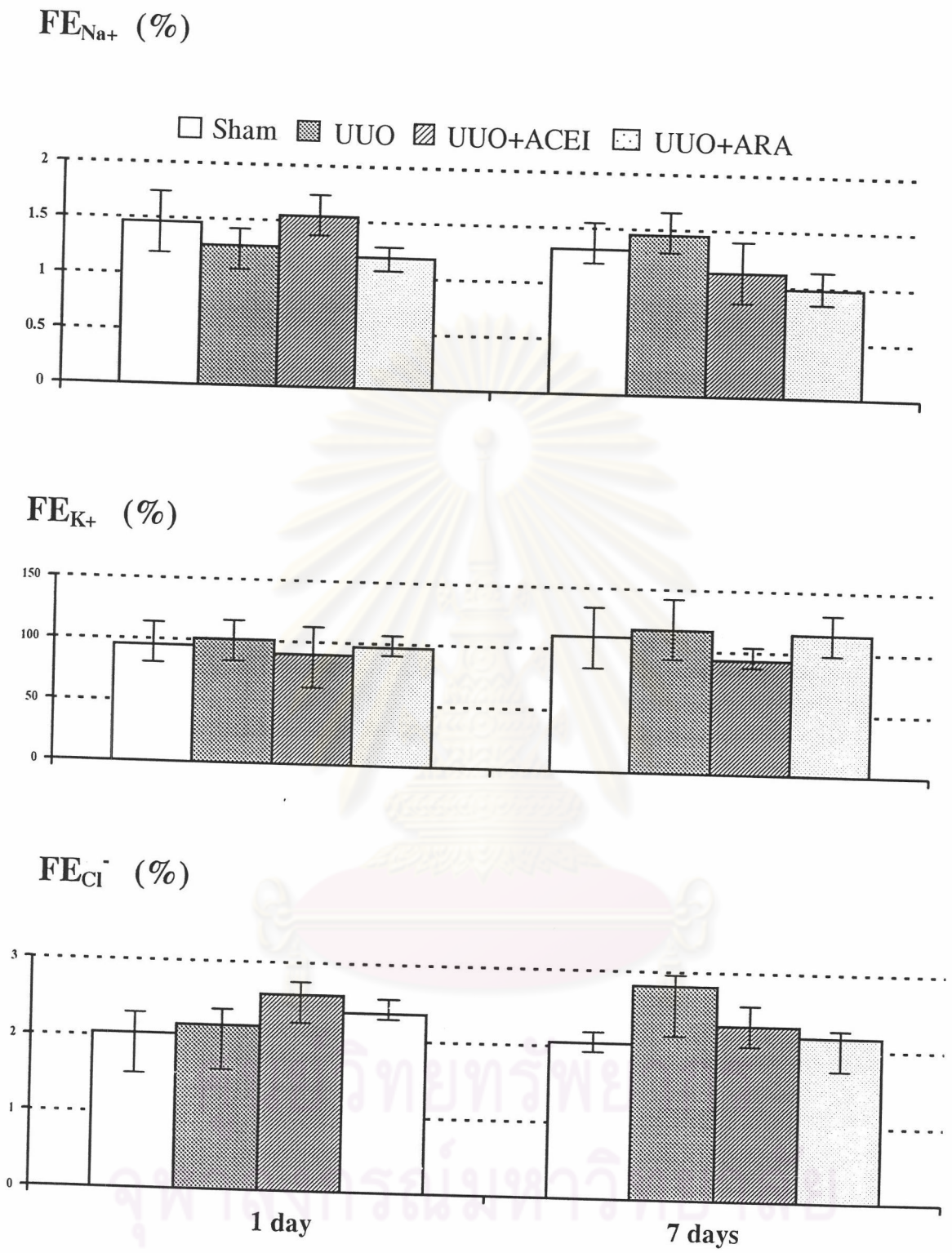

Figure 15 Fractional excretion of Na^+, K^+, and Cl^- in Sham, UUO, UUO+ACEI, and UUO+ARA groups from 1 day and 7 days after UUO (n = 8±1/group).

Table 3 Serum levels of Na^+, K^+, Cl^-, Cr, C_{Cr}, and BUN in Sham, UUO, UUO+ACEI, and UUO+ARA rats on day 1 or day 7 after UUO (Mean ± S.E.; n = 8 ± 1/group).

Serum levels	Groups / Duration period after UUO	Sham	UUO	UUO + ACEI	UUO + ARA
Na^+ (mmol/L)	1 day	143.00±2.73	140.50±2.06	135.75±1.29	141.37±3.46
	7 days	141.75±.47	140.87±0.29	138.87±0.44	139.50±0.40
K^+ (mmol/L)	1 day	3.90±0.24	3.48±0.07	4.03±0.16	4.22±0.16
	7 days	4.35±0.11	3.63±0.11	4.17±0.14	4.18±0.11
Cl^- (mmol/L)	1 day	97.75±3.14	91.75±2.10	99.25±1.53	93.50±3.65
	7 days	103.75±1.36	104.25±2.14	102.00±3.05	100.87±2.64
Cr (mg%)	1 day	0.57±0.06	0.62±0.02	0.65±0.03	0.65±0.01
	7 days	0.52±0.02	0.67±0.01	0.62±0.05	0.58±0.01
C_{Cr} (ml/min/100gBW)	1 day	0.70±0.11	0.58±0.08	0.80±0.08	0.47±0.08
	7 days	0.57±0.11	0.74±0.08	0.53±0.08	0.69±0.08
BUN (mg%)	1 day	25.00±2.48	30.62±1.36	31.25±2.05	31.75±2.08
	7 days	26.00±1.25	35.87±1.02	32.87±2.40	30.62±2.31
V (ml/min)	1 day	0.012±0.002	0.012±0.001	0.012±0.002	0.012±0.001
	7 days	0.017±0.002	0.013±0.001	0.013±0.001	0.016±0.001

(UUO = unilateral ureteral obstruction, ACEI = angiotensin converting enzyme inhibitor, ARA = angiotensin II receptor type 1 antagonist, Cr = Creatinine, C_{Cr} = Creatinine clearance, BW = body weight, V = urine flow rate)